(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
30 August 2001 (30.08.2001)

PCT

(10) International Publication Number
WO 01/62276 A1

(51) International Patent Classification[7]: A61K 38/27

(21) International Application Number: PCT/KR01/00170

(22) International Filing Date: 6 February 2001 (06.02.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
2000-8116 21 February 2000 (21.02.2000) KR

(71) Applicant *(for all designated States except US)*: **DAE-WOONG PHARM. CO., LTD.** [KR/KR]; 223-23, Sangdaewon-dong, Joongwon-ku, Sungnam, Kyunggi-do 462-120 (KR).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **YANG, Jeong-Hwa** [KR/KR]; #858-1601 Sulak Apt., 1151-9 Sanbon-dong, Kunpo-shi, Kyunggi-do 435-040 (KR). **LEE, Jang-Won** [KR/KR]; 387-4, Yangjae-dong, Seocho-ku, Seoul 137-130 (KR). **SHON, Mi-Young** [KR/KR]; #805-1302 Hyundai Apt., Sinjeongmaeul, Suji-residential 2nd project, Suji-counti, Pungduckchun-ri, Yongin-city, Kyunggi-do 449-840 (KR). **KIM, Young-Jun** [KR/KR]; 498-64 Banghak-dong, Dobong-ku, Seoul 132-020 (KR).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/62276 A1

(54) Title: A STABLE COMPOSITION COMPRISING EPIDERMAL GROWTH FACTOR AS AN ACTIVE INGREDIENT

(57) Abstract: The present invention relates to a stable composition which comprises an epidermal growth factor (hereinafter referred to as "EGF") as an active ingredient and a carboxyvinyl polymer as a base. The present inventors have identified that the EGF preparation comprising EGF as an active ingredient and acidic polymer such as carboxyvinyl polymer as a base has significant stability as compared with the prior arts using the base such as cellulose based polymer or neutral polymer. Therefore, the composition according to the present invention is useful in eye formulations, topical formulations for the skin and cosmetic formulations and so on.

A STABLE COMPOSITION COMPRISING EPIDERMAL GROWTH FACTOR AS AN ACTIVE INGRDIENT

TECHNICAL FIELD

The present invention relates to a stable composition comprising epidermal growth factor (hereinafter referred to as "EGF") as an active ingredient. More specifically, the present invention relates to a stable composition which comprises EGF having a biological activity and a carboxyvinyl polymer capable of being significantly increased stability of EGF in an aqueous solution as a base.

EGF(known as urogastrone) is a polypeptide having a molecular weight of 6045 which consists of 53 amino acid residues and includes three of disulfide bonds. EGF is known as a wound healing agent for the skin and cornea and a gastric ulcer healing agent because it represents a good activity for stimulating mitosis of various cells including epidermal and messenchymal cells and growth thereof and controlling secretion of gastric acid. (US Patent No. 140998 ; Carpenter, *Experimental Cell Research*, 164:1-10, 1986).

Although EGF shows a good activity for simulating differentiation of epidermal cells in vitro, it is very difficult that topical formulation containing EGF is developed to treat wounds of the skin and cornea for the reason that EGF has only a little effect in treating wounds when it is clinically applied to wounds.

EGF is biologically unstable and physicochemically non-homogenous so that its healing effects are not sufficient and its decomposition products

may induce allergic reactions. Accordingly EGF cannot exhibit sufficient effects for treating wounds in an application to a living body. EGF is very unstable at the room temperature, particularly in the presence of moisture. Although a lag time is required about 8 to 12 hours for DNA synthesis on wounds, EGF has a very short half-life of about 1 hour not to get the desired effects. Furthermore, EGF is physicochemically denatured at the room temperature and even in the state of cold storage when it is stored for a long time. When EGF is applied on the skin, EGF loses biological activity resulting from denaturation, decomposition, condensation and precipitation of EGF due to proteolytic enzymes to exist in wounds (Manning et al., *Pharmaceutical Res.*, 6:903-917, 1989).

In order to overcome biological unstableness of EGF and provide its desired wound healing effect, it is reported that EGF is continuously applied on wounds during initial few days of treatment which are most important time for wounds healing so as to constantly maintain an effective level of EGF (Frankline et al., *J. Lab. Clin. Med.*,108:103-108, 1986). In this regard, some sustained EGF-releasing formulations have been studied, which can continuously provide EGF to wounds.

As a result, US Patent No. 4,944,948 discloses the EGF/liposome gel formulations which continuously provide EGF to wounds using neutral phospholipids, negative-charged phospholipids and cholesterol; and EP Publication No. 312208 discloses the aqueous formulation being able to continuously release EGF which comprises pharmaceutically acceptable various water-soluble or water-swellable polymer as a base. However, although the above-mentioned prior arts disclose the formulations which can

continuously release EGF for 12 hours or more, they are unsuitable for producing in industrial fields because these formulations are unstable in long-term storage. Therefore, it has been required that a biological activity of EGF is maintained for a long time and a physicochemical stability thereof such as purity and homogeneity as well in order to provide EGF sufficient wounds healing effect as a medicine.

As a method to maintain physicochemical stability of EGF and inhibit a decrease of EGF activity, EP Publication No. 205051 provides the pharmaceutical composition in the form of a cream for dermal and ophthalmic use, which comprises 0.0001 - 0.005% (w/w) of EGF, 1 - 10 % (w/w) of surfactants, 5 - 45 % (w/w) of fatty substances and 0.3 - 0.8 % (w/w) of preservatives. EP Publication No. 267015 and US Patent No. 4717717 provides the compositions containing EGF stabilized by an addition of a water-soluble cellulose derivative to EGF. Also EP Publication No. 398615 and US Patent No. 5130298 provide the methods for stabilizing EGF by mixing EGF with a pharmaceutically acceptable metal cation such as zinc which is capable of preventing the degradation of EGF in aqueous solution since EGF is ionically bound with zinc.

However, although the above-mentioned stabilizers are added, the stability of EGF is maintained for about two months at 4℃. Therefore, when the topical formulation of EGF for the skin is clinically applied to wounds, they are unsuitable for utilizing in industrial fields since they have a little healing effect for wounds and the reduced stability of the formulation.

Accordingly, it is very desirable to develop the formulated preparation of EGF useful for treating incurable pathology and so on such as dermal ulcer

or corneal injure in the state of no special treating agent, which sufficiently exhibit the wound-healing effects, has a protected EGF against a loss of biological activity and quickly delivers EGF from the carrier to wounds when it is applied.

Thus, the present inventors have conducted numerous studies to develop the topical preparation of EGF which has a sufficient wound-healing effect and a good stability. As a result, we have found that the topical preparation comprising EGF as an active ingredient and acidic polymer such as carboxyvinyl polymer as a base can exhibit the desired good wound-healing effect and significant stability as compared with the prior arts using a base such as cellulose based polymer or neutral polymer.

DISCLOSURE OF THE INVENTION

It is therefore an object of the present invention to provide a biologically and physicochemically stable composition containing EGF, which comprises EGF as an active ingredient and a carboxyvinyl polymer as a base.

The composition according to the present invention comprises EGF as an active ingredient and a carboxyvinyl polymer as a base. EGF as an active ingredient may be isolated from natural sources or produced using recombinant DNA techniques. The content of EGF in the composition is within the range of 0.001 to 1,000μg/g on the basis of the total weight of the preparation, preferably in the range of 0.1 to 100μg/g such that EGF is pharmacologically effective. The pH of the composition according to the present invention is preferably in the range of 4 to 8, more preferably in the

range of 5 to 7 in order to keep EGF dissolved without denaturation.

A carboxyvinyl polymer which is used as a base in the present invention is a homopolymer having molecular weight of 1×10^6 to 4×10^6. The carboxyvinyl polymer, which is a cross-linked product of acrylic acid and aryl sucrose, is an acidic polymer indicating pH of 2.5 to 3.0 when it is dispersed in 1% aqueous solution. It has the wide range of viscosity even in a low concentration of less than 1% so that it is widely used as a base to suspension for oral, lotion, cream and gel preparation. Furthermore, the carboxyvinyl polymer contains carboxylic residue in the ratio of 56.0 to 68.0% regardless of a kind of polymer including Carbomer 934, Carbomer 934P, Carbomer 940, Carbomer 941 or Carbomer 947P. The content of carboxyvinyl polymer is within the range of 0.001 to 50 wt% on the basis of the total weight of the composition, preferably 0.005 to 25wt%, more preferably 0.01 to 10wt%.

The composition according to the present invention may further contain pharmaceutically acceptable additives, for example stabilizer, excipient, isotropic agent, moisturizing agent, pH controlling agent and so on.

The present inventors have conducted the stability test comparing the EGF preparation containing the carboxyvinyl polymer according to the present invention with EGF preparations containing another polymers as a base for six months at 4℃ and 25℃. In this case, EGF dissolved in 10mM phosphate buffer is used as a control and the content of EGF is analyzed with ELISA method. As a result, EGF preparation containing the carboxyvinyl polymer as a base according to the present invention shows a significant stabilization in the various concentration as compared with EGF preparations containing

another base as well as EGF dissolved in phosphate buffer. From this result, it is identified that EGF in EGF preparation according to the present invention is stabilized by the addition of the carboxyvinyl polymer regardless of contents thereof and then the polymer may be used as a base controlling its viscosity optionally and be added as a stabilizer depending on the purpose for use.

The composition containing EGF according to the present invention is useful in eye formulations, topical formulations for a skin such as cream, ointment, gel, patch and so on, and the composition may be used by coating or spreading on the cotton plane surface gauze, and the composition can be stored in a lyophilized form and then dissolved in a suitable solvent when it is used if necessary. Furthermore, the topical formulation for the skin may be useful in cosmetic formulation.

The present invention is more specifically explained by the following examples. However, it should be understood that the present invention is not limited to these examples in any manner.

Example 1

An eyedrop formulation containing Carbomer(0.1%)

EGF	0.5mg
Carbomer 934P	0.1g
Mannitol	5g
Methyl paraoxybenzoate	0.04g
Propyl paraoxybenzoate	0.01g
Sodium hydroxide	q.s
Distilled water for injection	q.s
Total	100g

The formulation was prepared by using the above-mentioned components in given amounts according to a conventional method. Specifically, mannitol, methyl paraoxybenzoate and propyl paraoxybenzoate were dissolved in appropriate amounts of distilled water for injection, Carbomer 934P(BFGoodrich, U.S.A.) was added to the solution and dispersed therein with stirring. Then, the solution was sterilized after controlling pH with sodium hydroxide, and mixed with filtered and sterilized solution of EGF(Daewoong Pharm., Korea) in distilled water for injection to obtain 100g of formulation.

Example 2

10mM of phosphate buffer containing EGF

EGF	0.5mg
Sodium hydrogen phosphate	0.14g
Sodium chloride	0.88g
20% phosphoric acid	q.s
Total	100g

The formulation was prepared by using the above-mentioned components in given amounts according to a conventional method. Specifically, sodium hydrogen phosphate and sodium chloride were dissolved in appropriate amounts of distilled water for injection, the solution was sterilized after controlling pH with 20% phosphoric acid, and mixed with filtered and sterilized solution of EGF in distilled water for injection to obtain 100g of formulation.

Example 3

An eyedrop formulation containing sodium carboxylmethylcellulose (0.5%)

EGF	0.5mg
Sodium carboxylmethylcellulose(Sod. CMC)	0.5g
Sorbitol	5.47g
Methyl paraoxybenzoate	0.05g
Sodium hydroxide	q.s
Distilled water for injection	q.s

Total	100g

The formulation was prepared by using the above-mentioned components in given amounts according to a conventional method. Specifically, sorbitol and methyl paraoxybenzoate were dissolved in appropriate amounts of distilled water for injection, sodium carboxylmethylcellulose was added to the solution and dispersed therein with stirring. Then, the solution was sterilized after controlling pH with sodium hydroxide, and mixed with filtered and sterilized solution of EGF in distilled water for injection to obtain 100g of formulation.

Example 4

A topical gel formulation containing Carbomer(1%)

EGF	5mg
Carbomer 934P	1g
Methyl paraoxybenzoate	0.2g
Propylene glycol	20g
Sodium hydroxide	q.s
Distilled water for injection	q.s
Total	100g

The formulation was prepared by using the above-mentioned components in given amounts according to a conventional method. Specifically, methyl paraoxybenzoate was dissolved in appropriate amounts of distilled water for injection, Carbomer 934P was added to the solution and

dispersed therein with stirring. Then, the pH of the solution was controlled with sodium hydroxide, the solution was blended with propylene glycol and sterilized by heating. Then, filtered and sterilized solution of EGF in distilled water for injection was added thereto to obtain 100g of formulation.

Example 5

A topical formulation containing Poloxamer(15%)

EGF	5mg
Poloxamer 407	15g
Methyl paraoxybenzoate	0.2g
Sodium hydrogen phosphate	272.18mg
Sodium chloride	666.22mg
Phosphoric acid	q.s
Propylene glycol	20g
Distilled water for injection	q.s
Total	100g

The formulation was prepared by using the above-mentioned components in given amounts according to a conventional method. Specifically, phosphate buffer was prepared by using sodium hydrogen phosphate, sodium chloride and phosphoric acid in given amounts. Methyl paraoxybenzoate as the preservative was dissolved to the phosphate buffer. Poloxamer 407(BASF, Germany) was added to the solution and dispersed therein with stirring. Then the solution was blended with propylene glycol,

and then EGF as the active ingredient was added thereto to obtain 100g of the formulation.

Example 6

A cream formulation containing Carbomer(0.1%)

EGF	0.05mg
Glycerin	4.5g
Methyl paraoxybenzoate	0.15g
Propyl paraoxybenzoate	0.05g
Carbomer 940	0.1g
Steary alcohol	1.75g
Cetyl alcohol	4.00g
Span #60	0.50g
Polyoxyl #40 stearate	2.00g
Triethanolamine	q.s
Distilled water for injection	q.s
Total	100g

The formulation were prepared by using the above-mentioned components in given amounts according to a conventional method. Specifically, glycerin and methyl paraoxybenzoate were dissolved in appropriate amounts of distilled water for injection, Carbomer 940(BF Goodrich, U.S.A.) was added to the solution and dispersed therein with stirring. Then, propyl paraoxybenzoate and the others were added to the

solution and emulsified with melting. Then, the solution was sterilized after controlling pH with triethanolamine, and mixed with filtered and sterilized solution of EGF(Daewoong Pharm., Korea) in distilled water for injection to obtain 100g of formulation.

Example 7

An ointment formulation containing Carbomer(0.1%)

EGF	0.5mg
Methyl paraoxybenzoate	0.10g
Propyl paraoxybenzoate	0.05g
Carbomer 940	0.1g
Beeswax	5g
Mineral oil	45g
Borax	0.2g
Microcrystalline wax	7.00g
Paraffin wax	10g
Distilled water for injection	q.s
Total	100g

The formulation was prepared by using the above-mentioned components in given amounts according to a conventional method. Specifically, methyl paraoxybenzoate, propyl paraoxybenzoate and Carbomer 940(BF Goodrich, U.S.A.) were dissolved and dispersed in appropriate amounts of distilled water for injection. The rest waxes were added to the

solution and emulsified at an elevated temperature. Then, the solution was sterilized by emulsifying, and mixed with filtered and sterilized solution of EGF(Daewoong Pharm., Korea) in distilled water for injection to obtain 100g of formulation.

Example 8

A patch formulation containing Carbomer(1%)

EGF	1.0mg
Polyvinylalcohol	20g
Polyvinylpyrrolidone	15g
Carbomer 940	1g
Polyethyleneglycol 4000	5g
Glycerol	3g
Distilled water for injection	q.s
Total	100g

The formulation was prepared by using the above-mentioned components in given amounts according to a conventional method. Specifically, Carbomer 940(BF Goodrich, U.S.A.), polyvinylalcohol, polyvinylpyrrolidine, PEG 400, Glycerol were dissolved and dispersed in appropriate amounts of distilled water for injection. The solution was sterilized at an elevated temperature, and mixed with filtered and sterilized solution of EGF (Daewoong Pharm., Korea) in distilled water for injection to obtain 100g of formulation. Then, the solution was pour into the mold to form

the patch.

Experiment 1

Stability test of eyedrop formulation

The stability of eyedrop formulation containing Carbomer prepared in Example 1 was tested as compared with the carboxyl methyl cellulose-containing formulation prepared in Example 2 which was known to stabilize EGF. The test was conducted to estimate EGF contents of each formulation with the lapse of time(2, 4, 8 and 18 weeks) under storage at 4℃ and 25℃. The sample of Example 2 dissolved in 10mM phosphate buffer was used to standard sample and the content of EGF was estimated by ELISA Method of Quantikine EGF ELISA kit(R&D, U.S.A).

Table 1 shows the result regarding the stability of EGF-containing eyedrop formulation as compared with standard sample at 4℃ and Table 2 shows the result regarding the stability of EGF-containing eyedrop formulation as compared with standard sample at 25℃.

As can be seen from the below Table 1, EGF content in phosphate buffer was decreased by about 10% in 8 weeks at 4℃ while EGF contents in Carbomer and carboxyl methyl cellulose were not changed until 8 weeks. However, in storage of 18 weeks at 4℃ condition, EGF contents in phosphate buffer and Carbomer formulation were not changed but EGF content in the carboxyl methyl cellulose was decreased to 87.3% in 18 weeks.

Table 1

Sample	Initial conc.(%)	conc.(%) at 4℃			
		2 weeks	4 weeks	8 weeks	18 weeks
Example 1 0.1% Carbomer	100±2.5	99.2±3.2	102.0±4.3	103.7±1.2	101.6±3.5
Example 2 10mM phosphate buffer	100±1.9	98.4±5.4	96.8±14.0	91.6±10.3	92.5±5.9
Example 3 0.5% sodium carboxyl methyl cellulose	100±2.1	104.9±3.4	99.7±6.0	102.7±2.3	87.3±3.1

As can be seen from the below Table 2, when the same formulations were stored at 25℃, the content of EGF in phosphate buffer sample was decreased by about 20% in 2 weeks and the content of EGF was continuously decreased after 4 weeks in the case of carboxyl methyl cellulose. However, the content of EGF in the formulation of Example 1 was little changed until 8 weeks. Also, when the formulation of Example 1 was stored for 18 weeks at the room temperature, the content of EGF was decreased by about 13% only. Therefore, it was identified that EGF stability was significantly increased even under storage at the room temperature in case of formulation containing Carbomer.

Table 2

Sample	Initial conc.(%)	conc.(%) at 25℃			
		2 weeks	4 weeks	8 weeks	18 weeks
Example 1 0.1% Carbomer	100±2.5	98.2±2.5	101.8±2.4	101.8±2.4	87.6±5.2
Example 2 10mM phosphate buffer	100±1.9	81.6±3.6	88.4±6.9	81.3±1.7	72.5±3.3
Example 3 0.5% sodium carboxyl methyl cellulose	100±2.1	93.5±6.5	88.4±0.2	78.5±2.7	48.7±9.3

Experiment 2

Stability test of topical gel formulation

The stability of topical gel formulation prepared in Example 4 was tested as compared with the topical formulation containing Poloxamer being widely used as a base for topical formulation which is a neutral polymer and is known to contribute to stabilization of protein resulting from lowering dielectric constant in an aqueous solution. The test was conducted to estimate EGF content of each formulation in storage in 18 weeks at 4℃ and 25℃. The sample dissolved in 10mM phosphate buffer was used to standard sample and the content of EGF was estimated by ELISA Method of Quantikine EGF ELISA kit(R&D, U.S.A).

Table 3 and 4 show the stability of each topical gel formulation at 4℃ and 25℃ respectively. As can be seen from the below Table 3, EGF content of the formulations containing Carbomer or Poloxamer was not changed until 8 weeks in cold storage. However, in storage for 18 weeks, EGF content of

Poloxamer-containing formulation was decreased by about 10%. As can be seen from the below Table 4, EGF content of 1% Carbomer-containing formulation was little changed until 18 weeks while EGF content of Poloxamer-containing formulation or phosphate buffer formulation was decreased by about 20% in 8 weeks and then continuously decreased until 18 weeks. The degree of decrease was further large in the case of Poloxamer-containing formulation. As seen from the eyedrop formulation, when a polymer was used as a base, the content of EGF was further decreased rather than phosphate buffer as time passed because the polymer might further promote the degradation of EGF in long-term storage. In conclusion, it was identified that the stability of EGF in formulation could be improved by using Carbomer as a base necessarily.

Table 3

Sample	Initial conc.(%)	conc.(%) at 4℃			
		2 weeks	4 weeks	8 weeks	18 weeks
Example 2 10mM phosphate buffer	100±1.9	98.4±5.4	96.8±14.0	91.6±10.3	92.5±5.9
Example 4 1% Carbomer	100±1.8	104.5± 14.2	102.3±2.6	101.2±0.8	100.3±2.3
Example 4 15% Poloxamer	100±2.8	103.5±9.3	95.7±0.8	94.2±4.2	90.5±4.5

Table 4

Sample	Initial conc.(%)	conc.(%) at 25℃			
		2 weeks	4 weeks	8 weeks	18 weeks
Example 2 10mM phosphate buffer	100±1.9	81.6±3.6	88.4± 6.9	81.3±1.7	72.5±3.3
Example 4 1% Carbomer	100±1.8	107.3± 2.0	92.5± 0.5	101.8± 2.4	99.5±4.5
Example 5 15% Poloxamer	100±2.8	90.3± 41.4	79.5± 5.0	78.5±2.7	66.4±2.6

Experiment 3

Stability test of cream, ointment and patch formulations

To estimate the stability of Carbomer-containing formulations prepared in Examples 6, 7, and 8, the test was conducted to estimate EGF content of each formulation with the lapse of time(2, 4, 8 and 18 weeks) under storage at 4℃ and 25℃. The sample of Example 2 dissolved in 10mM phosphate buffer was used to standard sample and the content of EGF was estimated by ELISA Method of Quantikine EGF ELISA kit(R&D, U.S.A).

Table 5 and 6 shows the stability of each cream, ointment and patch formulation at 4℃ and 25℃ respectively. As can be seen from the below Table 5, EGF content was not changed in cold storage. As can be seen from the below Table 6, EGF content was little changed at a room temperature. Therefore, it was identified that the stability of EGF in the formulation could be improved by using Carbomer as a base regardless of the type of formulation.

Table 5

Sample	Initial conc.(%)	conc.(%) at 4℃			
		2 weeks	4 weeks	8 weeks	18 weeks
Example 6 0.1% Carbomer cream	100±2.9	99.6± 5.2	102.5± 7.2	101.4±1.9	97.9±6.4
Example 7 0.1% Carbomer ointment	100±2.3	97.0± 9.5	100.1± 5.7	98.9±2.1	98.5±3.3
Example 8 1% Carbomer patch	100±3.5	98.5± 6.5	97.4± 8.6	98.4±2.7	97.5±5.8

Table 6

Sample	Initial conc.(%)	conc.(%) at 25℃			
		2 weeks	4 weeks	8 weeks	18 weeks
Example 6 0.1% Carbomer cream	100±2.9	100.1± 6.2	100.5± 3.3	96.7±2.5	95.2±4.5
Example 7 0.1% Carbomer ointment	100±2.3	99.5± 6.3	102.1± 5.1	94.8±1.8	96.2±8.9
Example 8 1% Carbomer patch	100±3.5	100.2± 12.3	96.5± 9.4	97.2±8.8	95.7±8.4

As shown in the results obtained from the above experiments, the present invention provides a stable EGF composition, which comprises carboxyvinyl polymers as a base and biologically active EGF of which the stability is biologically and physicochemically ensured.

WHAT IS CLAIMED IS :

1. A stable composition which comprises a biologically active epidermal growth factor(hereinafter referred to as "EGF") as an active ingredient and a carboxyvinyl polymer as a base.

2. The stable composition according to Claim 1, wherein the biologically active EGF is isolated from natural sources or produced using recombinant DNA techniques.

3. The stable composition according to Claim 1, wherein the content of EGF is within the range of 0.001 to 1,000μg/g on the basis of a total weight of the preparation.

4. The stable composition according to Claim 1, wherein the content of EGF is within the range of 0.1 to 100μg/g on the basis of a total weight of the preparation.

5. The stable composition according to Claim 1, wherein the pH of the composition in an aqueous solution is within the range of 4 to 8

6. The stable composition according to Claim 1, wherein the carboxyvinyl polymer is selected from the group comprising Carbomer 934, Carbomer 934P, Carbomer 940, Carbomer 941 or Carbomer 947P.

7. The stable composition according to Claim 1, wherein the content of carboxyvinyl polymer is within the range of 0.001 to 50(w/w)% on the basis of a total weight of the composition.

8. The stable composition according to Claim 1, wherein the content of carboxyvinyl polymer is within the range of 0.005 to 25(w/w)% on the basis of a total weight of the composition

9. The stable composition according to Claim 1, wherein the content of carboxyvinyl polymer is within the range of 0.01 to 10(w/w)% on the basis of a total weight of the composition.

10. The stable composition according to Claim 1, which is an eye formulation.

11. The stable composition according to Claim 1, which is a topical formulation.

12. The stable composition according to Claim 11, which is a cream formulation.

13. The stable composition according to Claim 11, which is an ointment formulation.

14. The stable composition according to Claim 11, which is a gel formulation

15. The stable composition according to Claim 11, which is a patch formulation.

16. The stable composition according to Claim 11, wherein the composition is spreaded on the cotton plane surface or gauze.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR01/00170

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 A61K 38/27

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimun documentation searched (classification system followed by classification symbols)
IPC A61K

Documentation searched other than minimun documentation to the extent that such documents are included in the fileds searched
Korean Patents and Applications for Inventions since 1975

Electronic data base consulted during the internationl search (name of data base and, where practicable, search trerms used)
MEDLINE, CAS ON LINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Son, K H. and Kwon, C. H. 'Stabilization of human epidermal growth factor(hEGF) aqueous formulation' In Pharm. Res. 1995, Vol.12(3), p451-454	1-16
A	EP 0312208A (Ethicon Inc.) 19 Apr 1989 See the whole document	1-16
A	US 4717717 A (Ethicon Inc) 05 Jan 1988 See the whole document	1-16

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevence
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevence; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevence; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 MAY 2001 (16.05.2001)	16 MAY 2001 (16.05.2001)

Name and mailing address of the ISA/KR	Authorized officer
Korean Intellectual Property Office Government Complex-Daejeon, Dunsan-dong, Seo-gu, Daejeon Metropolitan City 302-701, Republic of Korea Facsimile No. 82-42-472-7140	CHO, Myung Sun Telephone No. 82-42-481-5605

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/KR01/00170

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0312208 A	19. 04. 1989	JP 2000112 A	05. 01. 1990
		US 5427778 A	27. 06. 1995
		US 5457093 A	10. 10. 1995
		US 5705485 A	06. 01. 1998
US 4717717 A	05. 01. 1988	EP 267015 B	02. 09. 1992
		JP 10167980 A	23. 06. 1998